

A METHOD AND COMPOSITION TO ELICIT AN EFFECTIVE AUTOLOGOUS  
ANTITUMORAL IMMUNE RESPONSE IN A PATIENT

1 BACKGROUND OF THE INVENTION

3 Claim of Priority

4 The present application is based on and a claim to priority is  
5 made under 35 U.S.C. Section 119(e) to the provisional patent  
6 application currently pending in the U.S. Patent and Trademark  
7 Office having Serial No. 60/391,674 and a filing date of June 26,  
8 2002.

10 Field of the Invention

11 The present invention relates in general to a method and a  
12 composition to elicit an effective antitumoral immune response in  
13 a patient, specific to his or her own tumor antigens (i.e. an  
14 autologous antitumoral immune response). More specifically, the  
15 present invention relates to a method to elicit an effective  
16 autologous antitumoral immune response in a cancer patient which  
17 comprises generating, preserving, and storing specific tumor  
18 associated antigens, and eliciting the autologous antitumoral  
19 immune response, at least in part, through a combination of dual  
20 vaccines. The present invention further provides for enhancement  
21 of the antitumoral immune response resulting from an internal  
22 vaccine and an external vaccine by activating antigen presenting  
23 cells, as well as by inhibiting a tolerance immune response in

1 cancer patients. The present invention further provides a method  
2 for preparing an autologous hemoderivative composition for  
3 utilization in the inventive method as an external vaccine.

#### 4 5 DESCRIPTION OF THE RELATED ART

6 Initially, immunotherapy techniques began with treatments or  
7 vaccines for the prevention of infectious diseases, and they have  
8 since virtually eradicated many such diseases. Generally,  
9 immunotherapy has exploited the ability of an immune system to  
10 respond when it is in contact with alien molecules known as  
11 antigens. An immune response is specifically addressed against  
12 antigenic molecules or against other organisms that express these  
13 antigenic properties, collectively known as antigens. When the  
14 antigen is not a living organism, the immune response is frequently  
15 mediated by cells identified as helper cells or CD4+ lymphocytes,  
16 and by antibody producing cells, the final effectors being  
17 antibodies, for example, immunoglobulin molecules that are  
18 circulating throughout a patient's bloodstream. When the antigen  
19 is a component of a live cell or microorganism, the immune response  
20 is mediated by circulating cells and also the effector cells,  
21 mainly, cytotoxic CD8+ lymphocytes.

22 Frequently, the presence of antibodies against a specific  
23 antigen can be tested by the immediate (20-60 minute) response  
24 obtained when a dermic test with the antigen is performed. The  
25 cellular response is tested by a delay (48-72 hour) response after

1 dermic exposure to the antigen. As a consequence, the antibodies  
2 and the cellular immune response to them are also known as  
3 immediate response or delay response, respectively.

4 Most of the time, the immune response is associated with  
5 cooperation between the antibodies, or the molecular mediated arm,  
6 and the cellular mediated arm. Typically, the cooperative response  
7 is the antibodies known dependent cytotoxic response. Classical  
8 immunotherapy techniques have used such antigens as vaccine agents.  
9 These agents were treated to avoid their pathogenicity and/or they  
10 were mixed with adjuvants in order to facilitate their  
11 accessibility, recognizance or stimulant activity. Antigens are  
12 necessary for immune response because, by definition, an immune  
13 response is a specific antigen-addressed response, however, modern  
14 research has recognized that sometimes although antigens are  
15 present, their immunological power is not enough to stimulate an  
16 effective immune response. In such cases, the immune response can  
17 be elicited by other substances or by modified antigens with more  
18 powerful antigenic activity and cross-reactivity with the specific  
19 target of the immune response. In addition, some agents have been  
20 identified which elicit immune responses not upon specific  
21 antigens, but, rather, upon specific or global reactive portions of  
22 the immune system. As a consequence, today it is more appropriate  
23 to identify this whole family of compounds which may be used in  
24 immunotherapy, including specific antigens and all other agents  
25 that elicit or enhance a response against antigens or from the

1 immune system, collectively, as immunogens.

2 It is appreciated that human cancer immunotherapy has been in  
3 use and has been subject of reported research for years. More in  
4 particular, human cancer immunotherapy began when specific antigens  
5 in malignant cells were recognized. With this knowledge, the  
6 stimulation of a patient's immune response against the specific  
7 antigens of these malignant cells as an antitumoral treatment was  
8 explored. Along with surgery, chemotherapy, and radiotherapy,  
9 immunotherapy provides yet another therapeutic technique available  
10 in Oncology. Frequently, these therapeutic techniques are employed  
11 simultaneously or successively in various treatment regimens.

12 Cancer immunotherapy techniques are commonly grouped into one  
13 of two categories, namely, non-specific immunotherapy or specific  
14 immunotherapy. The goal of non-specific cancer immunotherapy is an  
15 increase in all of a patient's immune responses, thereby improving  
16 the activity level throughout the patient's immune system.  
17 Specific cancer immunotherapy, on the other hand, has the goal of  
18 stimulating a singular antitumoral immune response that may be  
19 directed against the patient's tumor or the patient's tumor type or  
20 an antigen of that tumor.

21 Each of these immunotherapy techniques may be further grouped  
22 into sub-categories, being either an active immunotherapy or an  
23 adoptive immunotherapy. Active immunotherapy techniques comprise  
24 methods wherein the immune response induced by treatment is  
25 dictated by the patient's own immune system, whereas, adoptive

1 immunotherapy techniques comprise methods in which one, several, or  
2 all of the components of the patient's immune system are replaced,  
3 thereby dictating an alternate immune response.

4 Thus, there are in actuality, four commonly known  
5 immunotherapy techniques utilized in the field of cancer treatment,  
6 specifically: active non-specific immunotherapy; adoptive  
7 non-specific immunotherapy; active specific immunotherapy; and,  
8 adoptive specific immunotherapy. Numerous agents have been  
9 produced, modified, or protocolized by different methods in order  
10 to be employed as immunogens in one or more of these four cancer  
11 immunotherapy techniques. Each these techniques, however, exhibit  
12 certain shortcomings which hinder their development and limit their  
13 implementation as effective and safe cancer treatment regimens,  
14 except for short periods of time and only for a small number of  
15 select tumor types. A description of each of these four cancer  
16 immunotherapy techniques is presented below in further detail,  
17 including the known shortcomings of each.

18 Active non-specific immunotherapy includes the administration  
19 of a biological or chemical agent that has been proven to stimulate  
20 immune system activity. Compositions comprising bacille Calmette-  
21 Guerin(BCG), *Corynebacterium*, levamisole, and zinc compounds have  
22 been among the most tested immunogens. The basic supposition is  
23 that cancer patients are always immune-depressive and that this  
24 technique could restore the immune system activity, including  
25 antitumoral response. It must be noted, however, that the supposed

1 global depression of the immune system is yet to be demonstrated in  
2 most cancer patients, thus, the global immune-restoration is not  
3 necessarily a proper goal of treatment, and the possible secondary  
4 and potentially negative effects of non-specific immunotherapy are,  
5 therefore, not justified. In fact, in some cases it has been  
6 reported that non-specific hyper-stimulation of the immune system  
7 has also produced enhancement or tumor progression. This result  
8 may be explained by the stimulation of cell populations with  
9 properties of tolerance or suppression.

10 Adoptive non-specific immunotherapy comprises one variation  
11 involving the transfer of immunocompetent cell precursors, known as  
12 source cells, from a donor to a receptor in order to allow their  
13 proliferation, thereby resulting in the quimeric regeneration of  
14 immune system cell populations. In particular, tests have been  
15 performed on the transfer of a defined sub-population of  
16 immunocompetent cells to determine if it may increase their  
17 function. A common and well known treatment regimen utilizing this  
18 technique is an allogenic bone marrow transplant. One of the main  
19 drawbacks of this technique is the prevalence of reactions (i.e.  
20 rejection) of the transplanted bone marrow by the host.

21 More recently, another variation of adoptive non-specific  
22 immunotherapy has been tested which employs the administration of  
23 select components of the immune system which are candidates to  
24 promote a more amplified immune response. For example, recombinant  
25 molecules that are normally mediators of immune response, such as

1 interferons and interleukins, have been the agents produced by  
2 genetic recombination and employed as immunogens in order to expand  
3 antitumoral responses. These compounds are known as biological  
4 response modifiers and they are active as antitumoral agents but  
5 only in a few specific types of tumors such as, renal cell  
6 carcinoma, melanoma, hairy cell leukemia, and non-Hodgkin's  
7 lymphoma. Additionally, even when used to treat these specific  
8 types of tumors, most of the benefits are partial and temporary.  
9 The problem with this technique appears to be that the  
10 rate-limiting step of antitumoral immune response and the target  
11 step of a biological response modifier acting in isolation are  
12 presently unknown. As a consequence, the effectiveness of any  
13 treatment with these agents is fortuitous, at best.

14 Adoptive specific immunotherapy is a method of treatment that  
15 uses lymphocytes which have previously been in contact with tumor  
16 cell antigens, either in vivo or in vitro. In addition, this  
17 immunotherapy technique uses recombinant monoclonal antibodies  
18 against specific molecular targets expressed by malignant tumor  
19 cells. The antecedents of this procedure are the treatment of  
20 infectious diseases with hyperimmune serum or immunoglobulins.  
21 Subsequently, in the field of cellular mediated immunity, the  
22 intent was to collect tumor infiltrating lymphocytes or dendritic  
23 cells and to re-inject them with previously known pulse activation  
24 or clonal expansion.

25 Currently, there is active development directed towards the

1 use of recombinant monoclonal antibodies directed against molecular  
2 tumor targets which represents a variation of adoptive specific  
3 immunotherapy. Components of tumor receptors such as H2-neu and  
4 CD20, which may be over-expressed in cells of some breast cancers  
5 and non-Hodgkin's lymphomas, respectively, are the most effective  
6 target for the monoclonal antibodies currently available for  
7 immunotherapy. The effectiveness of the treatment of patients  
8 having malignant diseases with monoclonal antibodies also appears  
9 to be more the exception than the rule. The remissions are  
10 frequently limited to only a fraction of patients treated and  
11 having tumors with the supposed antigenic target, and these  
12 remissions are generally only temporary.

13 The difficulties encountered with this immunotherapy technique  
14 appear to be that molecular changes or losses in the target of the  
15 transferred immune effector are very frequent due to malignant  
16 disease evolution. This is due, at least in part, because tumor  
17 cells exhibit a high rate of spontaneous mutation as a consequence  
18 of their high proliferative turnover and their high rate of  
19 mutation induced by oncological therapies. This results in an  
20 immunological escape mechanism which detracts from the  
21 effectiveness of this immunotherapy technique.

22 Lastly, active specific immunotherapy utilizes a vaccine  
23 comprising tumor specific antigens, known as neo-antigens or tumor  
24 associated antigens (TAA). The existence of TAA has been well  
25 recognized for some time. This immunotherapy technique includes



1 all vaccine treatments that include the administration of antigens  
2 as tumor cells, tumor extracts, or as purified molecular compounds  
3 extracted from tumors. In order to enhance the elicited immune  
4 response, different procedures have been tested including alternate  
5 methods of inoculation (e.g. intradermal, subcutaneous,  
6 intramuscular, or intravenous), as well as the use of different  
7 adjuvants (e.g. tumor cells with genetic engineering to secrete  
8 immune-modulating cytokines, antigen pulsed dendritic cells, mixed  
9 antigens with BCG, tumor peptide antigens combined with chaperone  
10 heat shock proteins, and hapten potentiation of antigens).

11 In the last decade, active specific immunotherapy techniques  
12 have been developed utilizing autologous systems, the goal being to  
13 obtain a more specific immune response against well-demonstrated  
14 tumor cell antigens specifically expressed by an individual tumor.  
15 This represents a significant advance in active specific  
16 immunotherapy because it allows customization of the immunotherapy  
17 to an individual antigen profile that is generated in a specific  
18 tumor by spontaneous and therapeutically associated gene tumor cell  
19 mutations, through the individual patient-tumor history. Clinical  
20 assessment of such active specific immunotherapy techniques,  
21 however, indicates that it has only produced effective results in  
22 the treatment of a few tumors and, once again, the results obtained  
23 are only partial and are only temporary.

24 The difficulties encountered in active specific immunotherapy  
25 techniques are mainly twofold. The first problem is related to the

1 basic nature of cancer itself. More in particular, the malignant  
2 cells derived from a normal patient are the patient's self-cells  
3 and, therefore, their molecular composition is not normally  
4 antigenic relative to the host (i.e. the patient's) immune system.  
5 The molecules in tumor cells that are unrecognizable as the  
6 patient's self-cells are products of etiological or therapeutical  
7 mutations and/or specific epigenetic structural modifications. The  
8 concentration of these antigenic compounds are typically low in  
9 most malignant tissues, and their antigenicity is further reduced  
10 because the antigens are normally stored within the malignant  
11 cells, far from the afferent immune system. Additionally, these  
12 stored antigens are frequently destroyed by proteolysis when the  
13 malignant tumor cells die by programmed death or apoptosis, unless  
14 they are first protected, such as by protein induced cell stress.

15 The second difficulty encountered in active specific  
16 immunotherapy relates to the preparation of a vaccine having the  
17 patient's malignant tumor as its source. Here, both quantitative  
18 and qualitative limitations are present. To begin with, the number  
19 of inoculations and the amount of immunogen, or vaccine, in each  
20 inoculation as required by this technique are limited by the  
21 availability of surgical tumor specimens, and the typically weak  
22 antigens which are present at low cellular concentrations therein.  
23 In addition, and as noted above with respect to adoptive specific  
24 immunotherapy techniques, if tumor cells modify their antigenic  
25 profile due to their high rate of mutation, the immune effectors

1 elicited by inoculation of the original vaccine may not recognize  
2 a target in the remaining mutated tumor cells. As a result,  
3 repeated inoculations of the original vaccine will not usually be  
4 effective unless current surgical tumor specimens are available in  
5 order to prepare vaccines containing the successively mutated  
6 antigens, however, such current surgical tumor specimens are  
7 hardly, if ever, available.

8       Thus, it would be beneficial to provide a method to elicit an  
9 antitumoral immune response in a cancer patient and, more in  
10 particular, an effective autologous antitumoral immune response  
11 thereby providing a new, improved, and innovative active specific  
12 immunotherapy technique. Additionally, it would be helpful to  
13 provide a method to elicit such an effective autologous antitumoral  
14 immune response in a cancer patient via a treatment regimen  
15 structured to modify an antigen library of tumor cells, or TAA,  
16 thereby increasing the antigenicity relative to the patient's  
17 immune system. It would also be desirable for such an improved  
18 method to utilize a dual vaccine regimen including both an internal  
19 vaccine comprising the endogenous release of TAA from the tumor  
20 itself, as well as an external vaccine comprising a composition  
21 derived from an autologous blood specimen obtained from the patient  
22 at a plurality of discreet time periods over the course of the  
23 entire treatment regimen. Any such method would further benefit  
24 from the provision of a procedure to enhance the antitumoral immune  
25 response in a patient via the activation of an antigen presenting

1 cell or APC population, and to inhibit a tolerance immune response  
2 in the patient. In addition, a method for preparing a  
3 hemoderivative composition from an autologous blood specimen for  
4 use as an external vaccine would be desirable.

5  
6 SUMMARY OF THE INVENTION

7 In view of the many drawbacks inherent in the immunotherapy  
8 techniques currently known and used in the treatment of cancer, and  
9 as otherwise identified in the art, the present invention provides  
10 a method and a composition to elicit an antitumoral immune response  
11 in patients, thereby providing a new improved active specific  
12 technique for practicing cancer immunotherapy. It is noted that  
13 the method of the present invention comprises certain aspects of  
14 procedures known in medical practice and/or in medical research,  
15 such as treatment with cytokines, colony stimulating factors  
16 frequently used for hematological and immunological restoration,  
17 application of chemotherapy and indomethacin, which have been used  
18 and are the subject of continued research as antitumoral  
19 treatments, and the malignant cell autoschizis promoted by  
20 high-doses of ascorbic acid and menadione. Numerous other  
21 procedures employed by the present invention, however, are not  
22 known in the art, such as, by way of example only, an enhanced  
23 generation and subsequent in vivo storage of specific TAA in the  
24 tumor cells of a cancer patient, and a dual vaccine regimen  
25 including an internal vaccine comprising a release of previously

1 generated, preserved, and stored endogenous TAA from the tumor  
2 cells of the patient, and an external vaccine comprising an  
3 autologous hemoderivative composition. The procedures utilized by  
4 the method of the present invention, whether previously known or  
5 initially presented herein, utilize various compounds which are  
6 known in human pharmacology and approved for medical practice all  
7 over the world. The innovation of the present invention lies in  
8 the way these known compounds and procedures are utilized in  
9 combination with the inventive procedures presented herein to  
10 achieve the objectives of the present invention.

11 It is hereby asserted that the present invention defines an  
12 inventive method which allows the practice of a new, improved, and  
13 innovative autologous active specific immunotherapy technique. More  
14 importantly, the method of autologous active specific immunotherapy  
15 defined by the present invention is distinguishable from all  
16 previously known immunotherapy techniques, such as those described  
17 above. As an initial matter, it is noted that the method of the  
18 present invention provides for an enhancement of tumor antigenicity  
19 relative to an immune system of a cancer patient as a  
20 distinguishing factor in autologous active specific immunotherapy.  
21 Another distinguishing factor is that the inventive method  
22 comprises a dual vaccine, one being an internal vaccine and another  
23 being an external vaccine. As previously indicated, one vaccine is  
24 an internal vaccine because after the storage of one or more  
25 immunogens in the patient's tumor cells, the patient is

1 "vaccinated" by triggering the subsequent release of the immunogens  
2 (e.g. antigens, TAA, or vaccine) from the tumor cells to the  
3 interstitial spaces in the patient's body such as phagocytes,  
4 lymphatic vessels and/or blood vessels. The other vaccine is an  
5 external vaccine because the patient is vaccinated via a  
6 subcutaneous inoculation, or other inoculation technique, with a  
7 hemoderivative composition prepared from an autologous blood  
8 specimen containing the one or more immunogens (e.g. antigen, TAA,  
9 or vaccine).

10 As stated above, known autologous active specific  
11 immunotherapy techniques for cancer utilize a surgical specimen of  
12 a patient's tumor as a source of vaccine, which is another  
13 important distinction of the present invention. In particular, the  
14 known immunotherapy techniques which require surgical specimens of  
15 the patient's tumor inherently comprise as a limiting condition the  
16 availability of such a surgical specimen, which is rarely available  
17 more than once, and even then, it is more than likely during the  
18 initial stages of diagnosis and treatment. Therefore, it is not  
19 possible to update the vaccine when utilizing known immunotherapy  
20 techniques, as may be required if the remaining tumor changes its  
21 antigenic expression, which is not an uncommon occurrence given the  
22 high rate of mutation in such organisms.

23 Conversely, the present invention as described herein  
24 eliminates the need for surgical specimens of the patient's tumor,  
25 and rather utilizes the remnant tumor cells, and, more

1 specifically, the neo-antigens and/or TAA released from the remnant  
2 tumor cells into the patient's bloodstream which provide the source  
3 of immunogens for both an initial internal vaccine, as well as for  
4 subsequent internal and external vaccines. As a result, the  
5 present invention provides for a plurality of vaccines which may be  
6 repeatedly updated so as to be effective against the specific tumor  
7 antigens as they change. This is possible because the present  
8 invention utilizes the antigen library of the patient's remaining  
9 tumor to provide the immunogen which is the target of the immune  
10 response elicited from the internal vaccine and is subsequently the  
11 source of the antigenic immunogen in the blood utilized to produce  
12 an external vaccine. As such, the immunogen, and thus, the  
13 internal and external vaccines, are always contemporary each time  
14 the inventive method is employed.

15 As may be seen from the foregoing, the present invention  
16 comprises a new, improved, and inventive method of autologous  
17 active specific immunotherapy which, while incorporating certain  
18 aspects of known cancer immunotherapy techniques, comprises  
19 numerous novel features which eliminate many of the shortcomings of  
20 these previously known techniques. Furthermore, none of the novel  
21 features of the method of the present invention are anticipated,  
22 rendered obvious, suggested, or even implied by any known  
23 immunotherapy technique or other cancer treatment described herein  
24 or otherwise known.

25 Turning now to a further description of the method of the

1 present invention, it is generally directed towards eliciting an  
2 effective autologous antitumoral immune response in a cancer  
3 patient and comprises generating a plurality of neo-antigens or  
4 tumor associated antigens (TAA) in a plurality of tumor cells of  
5 the patient, preserving the plurality of TAA in the plurality of  
6 tumor cells, activating a plurality of antigen presenting cells  
7 (APC), breaking or inhibiting an immune tolerance response,  
8 triggering an internal vaccine in the patient, and providing the  
9 patient an external vaccine comprising an autologous hemoderivative  
10 composition. Additionally, the present invention comprises a  
11 method for the preparation of an autologous hemoderivative  
12 composition such as may be utilized in the foregoing method for  
13 eliciting an effective autologous antitumoral immune response, as  
14 well as the autologous hemoderivative composition. Additionally,  
15 the present invention comprises a method for performing an  
16 immunological assessment of an elicited immune response, as well as  
17 for performing a clinical assessment of an elicited antitumoral  
18 response.

19 To accomplish the objectives of the present invention, the  
20 method includes generating a plurality of neo-antigens or tumor  
21 associated antigens (TAA) in a plurality of tumor cells of the  
22 patient. The plurality of TAA may include peptides and/or proteins  
23 with molecular sites unrecognized as molecular components of the  
24 patient's self-cells and, therefore, of the normal organic  
25 composition, by the patient's immune system. These alien molecules



(i.e. TAA) are generated in malignant tumors, by genome abnormalities or mutations. The mutated genes can be attributed to etiopathogenesis of cancer (oncogenes, antioncogenes), physiopathology of cancer (high proliferation fraction with high rate of spontaneous mutations), or therapeutical interventions (radio- and chemo- induced mutations). Mutated genes can generate a plurality of TAA by their direct expression or by the promotion of intracellular conditions eliciting epigenetic normal protein transformation. In order to generate a plurality of TAA in tumor cells, it is necessary to increase in these cells their protein synthesis and mutation frequency.

Thus, the method also comprises inducing protein synthesis in a plurality of tumor cells by treating the patient with a suitable pharmaceutical compound in order to activate the growth factor-receptors, such as are typically highly expressed in most malignant cells. One pharmaceutical compound which is suitable for this purpose is insulin, due to the insulin-like growth factor-receptors which are highly expressed in many malignant cells.

Insulin action requires the agonism of a cellular insulin-receptor. As result of this agonism, the receptor is activated and several biological processes are started. Among the processes activated by insulin-receptor agonism are protein synthesis linked to the incorporation from outside the cell of amino acids. In addition, the known insulin-like growth factors (1 and 2) have their cell receptors, and their agonism promotes the

1 tumor cell growth and, therefore, the tumor cell protein synthesis.  
2 Insulin-like growth factors are very important in malignant growth,  
3 and most tumor cells have a high level of insulin-like growth  
4 factor-receptors. The cross-reactivity of insulin and insulin-like  
5 growth factors and their receptors is known. In particular,  
6 insulin promotes the protein synthesis mainly in tumor cells  
7 because it is the agonist of its own receptor but also it is  
8 cross-agonist of insulin-like growth factor-receptors highly  
9 expressed in most malignant cells as it was referred.

10 It is noted, however, that other pharmaceutical compounds may  
11 be suitable for use in the method of the present invention for  
12 inducing protein synthesis in tumor cells, and that such  
13 pharmaceutical compounds may be utilized either in combination with  
14 or as a substitute for insulin. Among the other pharmaceutical  
15 compounds known to exhibit insulin-like growth factors are,  
16 somatotrophin, estrogens, androgens, just to name a few, however,  
17 it is to be understood that any compound able to induce protein  
18 synthesis in tumor cells may be suitable for use in the method of  
19 the present invention.

20 In addition to inducing protein synthesis in a plurality of  
21 tumor cells of the patient, the present invention comprises  
22 generating chemical-induced gene mutations or epigenetic protein  
23 modifications in the plurality of tumor cells by treating the  
24 patient with DNA targeted chemotherapeutics, thereby resulting in  
25 the generation of a plurality of proteins unrecognizable as

1 self-proteins by the patient's immune system which, as previously  
2 indicated, are known as neo-antigens or tumor associated antigens  
3 (TAA).

4 Most of the compounds used in antitumoral chemotherapy include  
5 agents structured to avoid DNA synthesis, which is required for  
6 cell reproduction. In particular, these compounds may comprise  
7 agents acting upon the structures of the DNA double helix that  
8 avoids the kinetic or enzymatic activity in DNA duplication, for  
9 example, cyclophosphamide, or enzymatic inhibitors acting upon  
10 enzymes required for nucleotide antecessor synthesis, such as,  
11 fluorouracil, or enzymes required for recovery of nucleotide  
12 synthesis cofactors including such compounds as methotrexate.

13 All compounds used in antitumoral chemotherapy which interfere  
14 with the normal DNA sequence can induce punctual or sectorial  
15 mutations through the modification of polypeptide codification.  
16 The significance of these mutations is that the immunologic non  
17 self-recognizance by the patient's immune system is higher when  
18 further mutagenic events are induced. In the present invention, at  
19 least one, but preferably a plurality of such mutagenic drugs, or  
20 DNA targeted chemotherapeutics, may be utilized which are  
21 addressed with selectivity to the tumor cells. The selectivity of  
22 tumor cells is determined by the high level of expressed  
23 insulin-like growth factor-receptors, thereby allowing the DNA  
24 targeted chemotherapeutics to reach the malignant cells through  
25 the increased permeability and proliferative requirements induced

1 in these cells by the insulin.

2 In one alternate embodiment of the present invention, the  
3 method comprises promoting mutations in tumor cells via  
4 pharmacological agents and/or radiotherapeutical agents to produce  
5 chemical-induced or physical-induced gene mutations or epigenetic  
6 protein modifications, either in combination with or as a  
7 substitute for the aforementioned DNA targeted chemotherapeutics.

8 At least one embodiment of the method of the present invention  
9 further comprises at least temporarily preserving the plurality of  
10 TAA within the plurality of cells of the patient. In one preferred  
11 embodiment, the plurality of TAA is at least temporarily preserved  
12 in the plurality of malignant tumor cells of the patient, by  
13 promoting the synthesis of molecules which act as chaperones of  
14 such intracellular peptides and proteins. The method of the  
15 present invention thus further comprises the step of inducing the  
16 synthesis of stress shock protein (SSP). The SSP is known as a  
17 chaperone because it protects proteins, such as TAA, by generating  
18 molecular complexes with them, thereby masking their presence to  
19 the immune system of the patient, as well as other molecular  
20 aggressors such as proteases. The induction of SSP may be  
21 accomplished utilizing pharmacological agents which are similar,  
22 and in at least one embodiment, identical to those utilized for  
23 generating the plurality of TAA. Thus, in at least one  
24 embodiment, the method of the present invention may accomplish the  
25 dual objectives, generating TAA and inducing SSP, in a single step.

1 This is accomplished by the fact that the mechanisms involved in  
2 TAA generation, share the property of inducing SSP synthesis.  
3 Specifically, the present invention may employ the dual mechanisms  
4 of insulin hypoglycemia and chemotherapeutical induced stress.

5 More in particular, cells which are submitted to heat or other  
6 stress agents respond with the synthesis of a compound known as a  
7 heat shock protein (HSP) or, more generally, stress shock protein  
8 (SSP). The HSP or SSP have an inherent protective property for  
9 other cellular proteins or peptides by forming molecular complexes  
10 with them, at the risk, however, of the cellular proteins or  
11 peptides being denatured by the HSP or SSP. As the HSP or SSP form  
12 molecular complexes with these cellular proteins or peptides, the  
13 HSP and SSP are also commonly known as chaperones molecules.

14 In the method of the present invention, the plurality of tumor  
15 cells of the patient are exposed to such cellular stress via  
16 hypoglycemia and antitumoral chemotherapeuticals. As indicated  
17 above, this exposure is performed simultaneously with the  
18 generation of the plurality of TAA and, therefore, the chaperone  
19 molecules induced by the method preserve and at least temporarily  
20 store the plurality of TAA inside the plurality of tumor cells. In  
21 at least one embodiment, the method may also comprise administering  
22 indomethacin, cortisol derivatives, corticoid compounds, and other  
23 pharmacological agents to the patient to initiate the generation of  
24 SSP.

25 To elaborate further, when tumor cell stress is induced by

1 hypoglycemia through insulin treatment, it is noted that insulin,  
2 which may also utilized by the method of the present invention for  
3 inducing protein synthesis, in sufficient dosages produces  
4 hypoglycemia, which induces SSP synthesis in cells subjected to  
5 this glucose restrictive condition. Because malignant cells  
6 normally require an elevated level of glycolysis to begin with,  
7 hypoglycemia presents a particularly high level of risk for these  
8 cells and, therefore, a particularly high level of stress, with a  
9 subsequent high level of SSP or chaperone molecule synthesis which  
10 may be utilized to at least temporarily preserve and store the  
11 plurality of TAA in the plurality of malignant tumor cells.

12 In one further embodiment, the method of the present invention  
13 may utilize other pharmacological or nutritional treatments to  
14 compliment the insulin induced hypoglycemia, or as a substitute for  
15 insulin to induce this condition in the patient so as to stress the  
16 plurality of tumor cells, thereby accomplishing the objective of  
17 generating SSP or chaperone molecules to at least temporarily  
18 preserve and store the plurality of TAA in the tumor cells.

19 In one alternate embodiment, stress to the tumor cells may be  
20 chemically induced by DNA targeted chemotherapeutics. In  
21 particular, DNA targeted chemotherapeutics, similar to those  
22 described above for use in mutagenic TAA generation, are also known  
23 for inducing cell stress. Active metabolites of cyclophosphamide,  
24 5-fluorouracil, and methotrexate, are just a few of the drugs used  
25 in antitumoral chemotherapy which may also be employed by the

1 present invention to chemically stress the patient's tumor cells to  
2 induce generation of SSP or chaperone molecules. As previously  
3 indicated, the method of the present invention may employ these  
4 drugs for simultaneously generating TAA and SSP.

5 The above method for generating SSP may be optimized when  
6 conducted in conjunction with indomethacin, a drug which is very  
7 well known for other uses in medicine, and has been recognized as  
8 a promoter of SSP synthesis. Indomethacin is a positive modulator  
9 of DNA binding to heat shock translational factor (hstf-1). This  
10 factor, through DNA binding, starts and maintains SSP synthesis.

11 In one other alternate embodiment, the method of the present  
12 invention may utilize other pharmacological or radiotherapeutical  
13 agents to complement or as substitutes for the DNA targeted  
14 chemotherapeutics described above to chemically or physically  
15 stress tumor cells in the patient. Further, and as indicated  
16 above, indomethacin, cortisol derivatives, corticoid compounds, as  
17 well as other suitable pharmacologicals may be utilized in order to  
18 initiate SSP generation, thereby, enhancing the preserving and  
19 storing of the plurality of TAA in the plurality of tumor cells in  
20 the patient.

21 The method of the present invention further comprises the step  
22 of increasing the efficiency of the antitumoral immune response in  
23 cancer patients. More in particular, the presentation of an  
24 antigen to the immune system is facilitated by specific antigen  
25 presenting cells (APC), mainly to the lymphocytes, such

1 presentation being necessary to elicit an immune response. At the  
2 same time, however, the antitumoral efficiency of this response  
3 requires avoiding the eliciting of an immune tolerance response to  
4 the plurality of TAA.

5 To begin, activating a plurality of APC may be accomplished  
6 via an adequate cytokine treatment, such as by administering a  
7 granulocyte-macrophage colony stimulating factor (GM-CSF). Human  
8 recombinant GM-CSF is known as an immune modulating cytokine that  
9 increases the dendritic cell population promoting its maturation  
10 and, as consequence, it amplifies the dendritic cell function of  
11 antigen presentation in order to start the immune response. This  
12 pharmacological property has been used to potentiate cancer  
13 vaccines with different external immunogens. In the present  
14 invention, and in particular, in an internal vaccine as previously  
15 described, the GM-CSF activated plurality of APC encounter the  
16 plurality of TAA which was previously preserved and stored in the  
17 plurality of tumor cells of the patient's body, which have been  
18 subsequently released into the patient's bloodstream via the  
19 mechanisms of autoschizis and/or apoptosis, which are described in  
20 further detail below. Additionally, the GM-CSF activated plurality  
21 of APC may encounter the plurality of TAA contained in an external  
22 vaccine comprising an autologous hemoderivative composition, as is  
23 also discussed in greater detail below.

24 In one embodiment, other pharmacological or immunological  
25 agents or biological response modifiers may be utilized to further



1 increase the antitumoral immune response of GM-CSF, either as a  
2 complementary or substitutive methodological step.

3 In addition to increasing the encounters between the plurality  
4 of APC and the plurality of TAA, the method of the present  
5 invention further comprises breaking or inhibiting the immune  
6 tolerance response via pharmacological treatment and, in one  
7 preferred embodiment, by administering cyclophosphamide to the  
8 patient in a specific chronological sequence with the generation of  
9 the plurality of TAA.

10 Because the inventive method may be employed a plurality of  
11 times over the course of the patient's entire treatment regimen, it  
12 is necessary to minimize the immune tolerance response in the  
13 patient typically elicited by the immune-stimulation that has been  
14 described in cancer patients. Thus, the method of the present  
15 invention utilizes low dosages of cyclophosphamide in a specific  
16 chronological sequence with the antigenic stimulation to inhibit  
17 the immune tolerance response in the patient, prior to  
18 administration of both the internal vaccine and the external  
19 vaccine. It is to be understood that while the method of the  
20 present invention may utilize cyclophosphamide, it is not the  
21 exclusive means for breaking or inhibiting the immune tolerance  
22 response in the patient.

23 As indicated above, the present invention further comprises an  
24 internal vaccine. More specifically, the method comprises  
25 triggering the release of the plurality of TAA, which has been

1 preserved and stored in the plurality of tumor cells of the  
2 patient, via a pharmacological tumor cell death that preserves the  
3 immunogenicity of the plurality of TAA, or immunogenic cell death.

4 It is known that all chemotherapeutical treatments in oncology  
5 kill tumor cells by apoptosis, but the immunogenicity of such tumor  
6 cells is only preserved if these tumor cells are first exposed to  
7 a cellular stress prior to being killed. Therefore, although known  
8 antitumoral chemotherapy comprise a mechanism to induce tumor cell  
9 death, a preferred embodiment of the present invention comprises a  
10 mechanism for inducing pharmacological tumor cell death utilizing  
11 a mechanism of cellular body fragmentation via high dosages of  
12 ascorbic acid administered intravenously, which is known as  
13 autoschizis, which may or may not be potentiated with menadione  
14 administered to the patient simultaneously with the high dosages of  
15 ascorbic acid. Thus, the method of the present invention triggers  
16 the release of the plurality of TAA, preserved and stored in the  
17 plurality of tumor cells of the patient, into the patient's body  
18 via the various intracellular components through the interstitial  
19 space including, but not limited to phagocytes, lymphatic vessels  
20 and/or blood vessels, thereby allowing the plurality of TAA to  
21 encounter the plurality of APC, and the patient's immune system,  
22 thus initiating an autologous antitumoral immune response.

23 Following some controversy in the medical field with regard to  
24 the effects of ascorbic acid on terminal cancer patients, it has  
25 been demonstrated that ascorbic acid in high doses administered

1 intravenously is selectively cytotoxic for malignant cells and in  
2 high doses in vitro, it induces tumor cell death through a modified  
3 apoptosis mechanism known as autoschizis, as indicated above.  
4 Specifically, autoschizis is a cell death with fragmentation of the  
5 cell body and release of cell fragments and, more importantly with  
6 respect to the method of the present invention, the cell contents  
7 (i.e. the plurality of TAA) to the extracellular surroundings. As  
8 previously noted, other mechanisms of cell death, such as classical  
9 apoptosis, are only immunogenic if the cell has been exposed to the  
10 cellular stress prior to death, such that the antigens inside the  
11 cell are protected by chaperone compounds (e.g. SSP) induced by  
12 said stress, otherwise, the antigen may also be destroyed in the  
13 cell death process. The method of the present invention may  
14 comprise, in addition to the mechanism of cell death via  
15 autoschizis, a mechanism of cell death via chemotherapy-induced  
16 apoptosis, however, the apoptosis cell deaths will be immunogenic,  
17 because all of the tumor cells are exposed to cellular stress prior  
18 to death under the method of the present invention.

19 Currently, research is being conducted to determine the  
20 chemotherapeutic value of ascorbic acid in high dose administered  
21 intravenously to cancer patients, including the use of ascorbic  
22 acid potentiated by menadione. It is noted, however, that there  
23 are no known applications of ascorbic acid autoschizis, or any  
24 associated procedure, utilized to induce an antitumoral immune  
25 response or to start an immunotherapy treatment regimen. In the

1 method of the present invention, an internal vaccine is obtained by  
2 triggering the release of the plurality of TAA from the plurality  
3 of malignant cells of the patient, at least partially into the  
4 patient's bloodstream via autoschizis. As such, specimens of blood  
5 containing at least some of the released plurality of TAA may  
6 subsequently be utilized to prepare an external vaccine, as  
7 discussed below.

8 At least one alternate embodiment of the method of the present  
9 invention further comprises the use of menadione or another  
10 pharmacological agent to potentiate the mechanism of autoschizis  
11 and/or the use of chemotherapy and/or the use of radiotherapy in  
12 combination with or as a substitute for the intravenous  
13 administration of ascorbic acid to induce tumor cell death and the  
14 subsequent, immunogenic release of the plurality of TAA into the  
15 patient's system.

16 The method of the present invention further comprises  
17 administering an external vaccine to cancer patients, the external  
18 vaccine preferably comprising an autologous hemoderivative  
19 composition prepared from an autologous blood specimen containing  
20 at least some of the plurality of TAA released from tumor cells.

21 The present invention further provides a method for producing  
22 such an external vaccine. In particular, at least some of the  
23 plurality of TAA released into the patient's blood as molecular  
24 chaperone protected complexes as a result of the internal vaccine  
25 are distributed in blood cells and blood plasma where they may

1 become associated by external adhesion or by phagocytosis. When  
2 such a blood specimen is exposed to a hypotonic and hypothermic  
3 shock, essentially all of the plurality of TAA-chaperone complexes  
4 are released from the blood cells, into a supernatant. Afterwards,  
5 the supernatant may be exposed to thermal fractioning, such as by  
6 heating to approximately 100 degrees centigrade for approximately  
7 between 8 to 10 minutes. Under these conditions, the TAA-chaperone  
8 complexes are opened, and the plurality of TAA become free. In  
9 addition, under these conditions, a majority of the enzymatic and  
10 toxic properties of other molecules contained in the preparation  
11 are destroyed, but the immunogenic properties of the plurality of  
12 TAA is preserved. The method of the present invention further  
13 provides for filtration of the subsequent solution thereby  
14 resulting in the external vaccine comprising an autologous  
15 hemoderivative composition. The method of the present invention  
16 further provides for the inoculation of the patient, such as via  
17 subcutaneous injection, which is known for its efficiency to  
18 promote encounters between antigens and APC in other vaccination  
19 procedures.

20 A particular and significant advantage of preparing the  
21 external vaccine utilizing the method of the present invention is  
22 that the entire method requires minimal laboratory facilities,  
23 thereby providing a simple, safe, and economical method to prepare  
24 a vaccine, relative to those prepared in highly complex facilities  
25 where autologous biological specimens must be transported.

1           A more detailed description of the method for preparing an  
2 external vaccine comprising an autologous hemoderivative  
3 composition is as follows. The method of the present invention  
4 provides for extracting a blood specimen of approximately 20  
5 milliliters from a femoral artery of the patient into a first  
6 syringe pre-filled with approximately 5,000 international units  
7 (I.U.) of heparin having a concentration in a range of between  
8 approximately 250 to 300 I.U. per milliliter. The blood specimen  
9 solution is allowed to sediment or settle in vertical position at  
10 a temperature of approximately 37 degrees centigrade. After  
11 approximately one hour, an aliquot of a supernatant of white cell  
12 rich blood plasma is separated from the blood specimen solution  
13 into a second syringe containing between approximately 3 to 4 parts  
14 of distilled water per part of the plasma-cell layer forming a  
15 plasma-cell solution and, thereby, inducing a hypotonic cytolysis.  
16 The method of the present invention further provides that the  
17 plasma-cell solution be stored at approximately minus twenty  
18 degrees centigrade for a period of approximately 24 hours, after  
19 which, the plasma-cell solution is warmed up to approximately 37  
20 degrees centigrade in order to complete the hypotonic-hypothermic  
21 cytolysis process.

22           The resultant plasma-cell solution may be filtered through a  
23 glass wood membrane or optionally it is centrifuged at 2000 G, in  
24 order to clear the solution and remove gross precipitates. In yet  
25 another embodiment, the resultant plasma-cell solution may be

1     sonicated to clear the solution and remove gross precipitates. The  
2     resultant plasma-cell solution is then subjected to further thermal  
3     treatment. More in particular, the method of the present invention  
4     permits utilization of any one of a plurality of thermal treatments  
5     in order to obtain different immunogens. In one preferred  
6     embodiment, the plasma-cell solution is heated to approximately 100  
7     degrees centigrade for approximately between 8 to 10 minutes. The  
8     method also provides for allowing the solution to return to room  
9     temperature, approximately 25 degrees centigrade, until temperature  
10    equilibrium is reached. Finally, the resultant plasma-cell  
11    fraction may also be either filtered through glass wool membrane,  
12    centrifuged at 2000 G, or sonicated, followed by filtration through  
13    cellulose membranes ranging from between approximately 0.20 to 0.45  
14     $\mu\text{m}$  diameter.

15         While the method of the present invention for preparing an  
16    external vaccine presented above comprises one preferred  
17    embodiment, it is understood that alternative embodiments may be  
18    utilized to prepare an external vaccine comprising an autologous  
19    hemoderivative composition through modification of the methods for  
20    blood extraction, sedimentation, and/or specific temperatures and  
21    durations for thermal fractionation. In addition, it is understood  
22    that while a preferred embodiment of the present invention  
23    comprises subcutaneous inoculation of the patient with the external  
24    vaccine, inoculation via other mechanisms including, by way of  
25    example only, intradermal, intravenous, and/or intramuscular

1 vaccination are encompassed in the method of the present invention  
2 to elicit an efficient antitumoral immune response or an  
3 antitumoral biological response targeted to tumor cells, tumor  
4 stroma patient's immune system and/or molecular mediators of the  
5 host biological response against cancer disease.

6 Thus, from the foregoing, it is readily seen that the method  
7 of the present invention allows one to elicit an antitumoral immune  
8 response in a cancer patient which may be addressed against his or  
9 her own specific tumor. In addition, the method provides for the  
10 pharmacological management of a patient's own cancer cell's antigen  
11 library to increase a malignant tumor's antigenicity. Also, the  
12 method comprises releasing an internal autologous vaccine from a  
13 patient's own tumor(s), specific antigens eliciting an antitumoral  
14 immune response against the patient's remaining malignant cancer  
15 cells. A further aspect of the present invention is a method of  
16 preparing and providing an external autologous vaccine comprising  
17 a hemoderivative composition obtained at least in part from  
18 inducing the generation and subsequent release into a patient's  
19 bloodstream of tumor specific TAA. Yet another aspect of the  
20 present invention is a method to enhance the antitumoral immune  
21 response in a cancer patient by activating an APC population  
22 induced by cytokine treatment and inhibiting tolerance immune  
23 response in the patient. The present invention further provides a  
24 method for an immunologically assessing an immune response elicited  
25 by an autologous vaccine of a cancer patient via an intradermal



1 test, as well as a method for assessing an antitumoral response  
2 elicited by an autologous vaccine of the cancer patient. Most  
3 importantly, the method of the present invention provides an  
4 innovative and alternate technique for eliciting an antitumoral  
5 immune response in a cancer patient in the event that surgery,  
6 chemotherapy, radiotherapy, and/or other cancer treatment regimens  
7 fail.

8 These and other objects, features and advantages of the  
9 present invention will become more clear when the figures as well  
10 as the detailed description are taken into consideration.

#### 11 12 BRIEF DESCRIPTION OF THE DRAWINGS

13 For a fuller understanding of the nature of the present  
14 invention, reference should be had to the following detailed  
15 description taken in connection with the accompanying figures in  
16 which:

17 Figure 1 is a schematic view of one preferred embodiment of  
18 the inventive method to elicit an effective autologous antitumoral  
19 immune response in a patient.

20 Figure 2 is a schematic of the embodiment of Figure 1 further  
21 illustrating one preferred embodiment for generating and preserving  
22 a plurality of tumor associated antigens (TAA) in a plurality of  
23 cells in the patient.

24 Figure 3 is a schematic of the embodiment of Figure 1 further  
25 illustrating one preferred embodiment for activating a plurality of

1     antigen presenting cells (APC) in the patient.

2             Figure 4 is a schematic of the embodiment of Figure 1 further  
3     illustrating one preferred embodiment for inhibiting an immune  
4     tolerance response for the TAA in the patient.

5             Figure 5 is a schematic of the embodiment of Figure 1 further  
6     illustrating one preferred embodiment for triggering an internal  
7     vaccine in the patient.

8             Figure 6 is a schematic of the embodiment of Figure 1 further  
9     illustrating one preferred embodiment for preparing and providing  
10    an external vaccine to the patient.

11  
12    DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

13            While this invention is susceptible of embodiment in many  
14    different forms, there is shown in the figures and will herein be  
15    described in detail at least one specific embodiment, with the  
16    understanding that the present disclosure is to be considered as an  
17    exemplification of the principles of the invention and is not  
18    intended to limit the invention to the embodiment illustrated.

19            As indicated above, the present invention is directed in  
20    general to a new, improved, and innovative active specific  
21    immunotherapy technique. More in particular, the present invention  
22    is directed to a method and a composition to elicit an effective  
23    antitumoral immune response in a patient, specific to his or her  
24    own tumor antigens (i.e. an autologous antitumoral immune  
25    response). Thus, more specifically, the present invention is

1 directed to a method and composition to elicit an effective  
2 autologous antitumoral immune response in a cancer patient which  
3 comprises generating, preserving, and storing specific tumor  
4 associated antigens, and eliciting the autologous antitumoral  
5 immune response, at least in part, through a combination of dual  
6 vaccines. In addition, the present invention provides enhancement  
7 of the antitumoral immune response resulting from an internal  
8 vaccine and an external vaccine by activating antigen presenting  
9 cells, as well as by inhibiting a tolerance immune response in  
10 cancer patients. The present invention further provides a method  
11 for preparing a hemoderivative composition, and, more in  
12 particular, an autologous hemoderivative composition, for  
13 utilization in the inventive method as an external vaccine. Figure  
14 1 presents a schematic view of one preferred embodiment of the  
15 method of the present invention. More in particular, Figure 1  
16 illustrates one preferred embodiment of one complete treatment  
17 cycle of the method of the present invention. The method of the  
18 present invention may comprise completing a single treatment cycle,  
19 however, at least one embodiment of the present invention includes  
20 completing a plurality of treatment cycles.

21 To begin, the method of the present invention provides for  
22 generating a plurality of tumor associated antigens (TAA) in a  
23 plurality of cells of the patient. As indicated above, mutated  
24 genes can generate a plurality of TAA by their direct expression or  
25 by the promotion of intracellular conditions eliciting epigenetic

1 normal protein transformation. In order to generate the production  
2 of a plurality of TAA in tumor cells, it is necessary to increase  
3 in these cells their protein synthesis and mutation frequency.

4 Thus, at least one embodiment of the method of the present  
5 invention further comprises inducing protein synthesis in a  
6 plurality of tumor cells by treating the patient with a suitable  
7 pharmaceutical compound in order to activate the growth  
8 factor-receptors, such as are typically highly expressed in most  
9 malignant cells. One pharmaceutical compound which is suitable for  
10 this purpose is insulin, due to the insulin-like growth factor-  
11 receptors which are highly expressed in many malignant cells. In  
12 particular, insulin promotes the protein synthesis mainly in tumor  
13 cells because it is the agonist of its own receptor but also it is  
14 cross-agonist of insulin-like growth factor-receptors highly  
15 expressed in most malignant cells as it was referred.

16 It is noted, however, that other pharmaceutical compounds may  
17 be suitable for use in the method of the present invention for  
18 inducing protein synthesis in tumor cells, and that such  
19 pharmaceutical compounds may be utilized either in combination with  
20 or as a substitute for insulin. Among the other pharmaceutical  
21 compounds known to exhibit insulin-like growth factors are,  
22 somatotrophin, estrogens, androgens, just to name a few, however,  
23 it is to be understood that any compound able to induce protein  
24 synthesis in tumor cells may be suitable for use in an embodiment  
25 of the method of the present invention.

1           In addition to inducing protein synthesis in the plurality of  
2       cells of the patient, the present invention comprises generating  
3       chemical-induced gene mutations or epigenetic protein modifications  
4       in the plurality of tumor cells by treating the patient with DNA  
5       targeted chemotherapeutics, thereby resulting in the generation  
6       of a plurality of proteins unrecognizable as self-proteins by the  
7       patient's immune system which, as previously indicated, are known  
8       as neo-antigens or tumor associated antigens (TAA).

9           Many of the compounds used in antitumoral chemotherapy include  
10      agents structured to avoid DNA synthesis, which is required for  
11      cell reproduction. In particular, DNA targeted chemotherapeutics  
12      comprise agents which act upon the structures of the DNA double  
13      helix that avoid the kinetic or enzymatic activity in DNA  
14      duplication and include, but are not limited to, cyclophosphamide,  
15      or enzymatic inhibitors acting upon enzymes required for nucleotide  
16      antecessor synthesis, such as, fluorouracil, or enzymes required  
17      for recovery of nucleotide synthesis cofactors including such  
18      compounds as methotrexate. One embodiment of the present invention  
19      may comprise administering at least one of these DNA targeted  
20      chemotherapeutics to the patient during a preparatory treatment  
21      phase. In one preferred embodiment, as illustrated in Figure 2,  
22      the method of the present invention comprises administering a  
23      plurality of DNA targeted chemotherapeutics to the patient during  
24      the preparatory treatment phase.

25           Thus, the method of the present invention provides for

1 administering one or more compounds to the patient during a  
2 preparatory phase of the treatment cycle for generating a plurality  
3 of TAA. The compounds selected to be administered to the patient  
4 are among those known to induce protein synthesis, such as insulin,  
5 as well as to generate production of a plurality of tumor  
6 associated antigens (TAA) in a plurality of the cells, and in one  
7 preferred embodiment, the malignant tumor cells of the patient.

8 As illustrated in Figure 2, one preferred embodiment of the  
9 method of the present invention comprises administering insulin to  
10 the patient each day of the preparatory treatment phase or, more  
11 specifically, days one through four of the treatment cycle, at a  
12 daily dosage of approximately 0.3 international units (I.U.) per  
13 kilogram of body weight. In addition, Figure 2 also illustrates  
14 that in a preferred embodiment, a plurality of DNA targeted  
15 chemotherapeutics are administered to the patient during the  
16 preparatory treatment phase (e.g. days one through four of the  
17 treatment cycle). Specifically, one preferred embodiment of the  
18 method of the present invention comprises administering  
19 cyclophosphamide, at a daily dosage in a range of between  
20 approximately 100 to 200 milligrams, methotrexate, at a daily  
21 dosage in a range of between approximately 2.5 to 12.5 milligrams,  
22 and fluorouracil, at a daily dosage in a range of between  
23 approximately 125 to 250 milligrams, to the patient each day of the  
24 preparatory treatment phase. In at least one embodiment of the  
25 present invention, the preparatory phase comprises days one through

1 five of the treatment cycle.

2 At least one embodiment of the method of the present invention  
3 further comprises at least temporarily preserving the plurality of  
4 TAA within the plurality of cells of the patient. In one preferred  
5 embodiment, the plurality of TAA is at least temporarily preserved  
6 in the plurality of malignant tumor cells of the patient, by  
7 promoting the synthesis of molecules which act as chaperones of  
8 such intracellular peptides and proteins. The method of the  
9 present invention thus further comprises the step of inducing the  
10 synthesis of stress shock protein (SSP). The SSP is known as a  
11 chaperone because it protects proteins, such as TAA, by generating  
12 molecular complexes with them, thereby masking their presence to  
13 the immune system of the patient, as well as other molecular  
14 aggressors such as proteases. The induction of SSP may be  
15 accomplished utilizing pharmacological agents which are similar,  
16 and in at least one embodiment, identical to those utilized for  
17 generating the plurality of TAA. Thus, in at least one  
18 embodiment, the method of the present invention may accomplish the  
19 dual objectives of generating TAA and inducing SSP in a single  
20 step. This is accomplished by the fact that the mechanisms  
21 involved in TAA generation, are similar to those for inducing the  
22 synthesis of SSP. Specifically, the present invention may employ  
23 the dual mechanisms of insulin hypoglycemia and chemotherapeutical  
24 induced stress.

25 Thus, in the method of the present invention, the plurality of

1 tumor cells of the patient are exposed to cellular stress via  
2 hypoglycemia and antitumoral chemotherapeutics. As indicated  
3 above, this exposure is performed simultaneously with the  
4 generation of the plurality of TAA and, therefore, the chaperone  
5 molecules induced by the method preserve and at least temporarily  
6 store the plurality of TAA inside the plurality of tumor cells. In  
7 at least one embodiment, the method may further comprise  
8 administering indomethacin, cortisol derivatives, corticoid  
9 compounds, and other pharmacological agents to the patient to  
10 initiate the generation of the plurality of SSP.

11 As such, at least one embodiment of the present invention  
12 further comprises preserving the plurality of TAA by inducing the  
13 synthesis of a plurality of SSP. More in particular, the method of  
14 the present invention may include inducing the synthesis of the SSP  
15 comprises by administering indomethacin to the patient. In one  
16 alternate embodiment, the method of the present invention may  
17 include inducing the synthesis of the SSP by administering a  
18 corticoid compound to the patient.

19 Also, as indicated above, the method of the present invention  
20 further comprises storing the TAA in the plurality of cells of the  
21 patient by inducing the synthesis of a plurality of stress shock  
22 proteins (SSP). Once again, the method may comprise inducing the  
23 synthesis of the SSP comprises by administering indomethacin to the  
24 patient. In one alternate embodiment, the method of the present  
25 invention may include inducing the synthesis of the SSP by



1 administering a corticoid compound to the patient.

2 The method of the present invention also comprises breaking or  
3 inhibiting the immune tolerance response relative to the TAA  
4 generated in the cells of the patient to enhance the antitumoral  
5 immune response. In at least one embodiment, inhibiting the immune  
6 tolerance response is accomplished via pharmacological treatment  
7 and, in one preferred embodiment, by administering cyclophosphamide  
8 to the patient in a specific chronological sequence with the  
9 generation of the plurality of TAA.

10 Because the inventive method may comprise completing a  
11 plurality of treatment cycles over the course of the patient's  
12 entire treatment regimen, it becomes necessary to minimize the  
13 immune tolerance response in the patient typically elicited by the  
14 immune-stimulation that has been described in cancer patients.  
15 Thus, in at least one embodiment, the method of the present  
16 invention utilizes low dosages of cyclophosphamide to inhibit the  
17 immune tolerance response in the patient, prior to administration  
18 of both the internal vaccine and the external vaccine. It is to be  
19 understood that while the method of the present invention may  
20 utilize cyclophosphamide, it is not the exclusive means for  
21 breaking or inhibiting the immune tolerance response in the patient  
22 encompassed by and which may be utilized in conjunction with the  
23 method of the present invention.

24 In particular, one embodiment of the method of the present  
25 invention comprises administering cyclophosphamide to the patient

1 during a first intermediate phase of the treatment cycle. More  
2 specifically, and as illustrated in Figure 4, one preferred  
3 embodiment of the method of the present invention comprises  
4 administering cyclophosphamide at a dosage of approximately 300  
5 milligrams per square meter of surface area of the patient's body,  
6 on day five of the treatment cycle.

7 The method of the present invention further comprises  
8 activating a plurality of antigen presenting cells (APC) in the  
9 patient to further enhance the antitumoral immune response. More  
10 in particular, the presentation of an antigen to the immune system  
11 is facilitated by specific APC, mainly to the lymphocytes, and is  
12 necessary to elicit an immune response. At the same time, however,  
13 the antitumoral efficiency of this response requires avoiding the  
14 eliciting of an immune tolerance response to the plurality of TAA.

15 In at least one embodiment, activating a plurality of APC may  
16 be accomplished via an adequate cytokine treatment, such as, for  
17 example, administering a granulocyte-macrophage colony stimulating  
18 factor (GM-CSF). Human recombinant GM-CSF is known as an immune  
19 modulating cytokine that increases the dendritic cell population  
20 promoting its maturation and, as consequence, it amplifies the  
21 dendritic cell function of antigen presentation in order to start  
22 the immune response. In the present invention, and in particular,  
23 in conjunction with an internal vaccine as previously described,  
24 the GM-CSF activated plurality of APC encounter the plurality of  
25 TAA released into the patient's bloodstream via the mechanisms of

1     autoschizis and/or apoptosis, as previously described in detail.  
2     Additionally, the GM-CSF activated plurality of APC may encounter  
3     the plurality of TAA contained in an external vaccine comprising an  
4     autologous hemoderivative composition, as also discussed in further  
5     detail below. It is understood to be within the scope of the  
6     method of the present invention to administer alternate  
7     pharmacological or immunological agents or biological response  
8     modifiers to either increase the antitumoral immune response of  
9     GM-CSF, or as a substitute for GM-CSF.

10         In one preferred embodiment, the method of the present  
11     invention comprises activating a plurality of antigen presenting  
12     cells (APC), to further enhance the antitumoral immune response in  
13     the patient, by administering a cytokine to the patient during a  
14     primary treatment phase. In at least one embodiment, the method of  
15     the present invention includes administering the cytokine  
16     comprising granulocyte-macrophage colony stimulating factor (GM-  
17     CSF) to the patient. As illustrated in Figure 3, one preferred  
18     embodiment of the method of the present invention comprises  
19     administering GM-CSF to the patient on each day of the primary  
20     treatment phase at a daily dosage in a range of between  
21     approximately 150 to 250 micrograms. In at least one embodiment,  
22     the primary treatment phase of the method comprises day eight  
23     through twelve of the treatment cycle.

24         As discussed above in some detail, the method of the present  
25     invention further comprises the new and innovative feature of

1 triggering an internal vaccine in the patient. As disclosed  
2 herein, the internal vaccine comprises the release of the TAA  
3 previously generated, preserved, and stored in the plurality of  
4 tumor cells of the patient's body, via a tumor cell death that  
5 preserves the immunogenicity of the TAA, known as an immunogenic  
6 cell death.

7 In particular, the method of the present invention provides  
8 for triggering the release of the plurality of TAA into the  
9 patient's body via the various intracellular components through the  
10 interstitial space including, but not limited to phagocytes,  
11 lymphatic vessels and/or blood vessels, thereby allowing the  
12 plurality of TAA to encounter the plurality of APC, and the  
13 patient's immune system, thereby initiating an autologous  
14 antitumoral immune response. In at least one embodiment, the  
15 method of the present invention includes administering ascorbic  
16 acid to the patient during the primary treatment phase to induce  
17 immunogenic cell death through a modified apoptosis mechanism known  
18 as autoschizis. In one preferred embodiment, the internal vaccine  
19 is triggered via administering the ascorbic acid to the patient  
20 intravenously, for example, in a lactate-ringer solution.

21 As illustrated in Figure 5, a preferred embodiment of the  
22 present invention comprises triggering the internal vaccine by  
23 inducing autoschizis by administering ascorbic acid to the patient  
24 during each day of the primary treatment phase. More specifically,  
25 the preferred embodiment of the method includes administering the

1 ascorbic acid to the patient each day of the primary treatment  
2 phase at a daily dosage of approximately 25 grams in approximately  
3 250 milliliters of a lactate-ringer solution. As noted above, the  
4 ascorbic acid is preferable administered intravenously. As also  
5 noted above, in at least one embodiment of the method of the  
6 present invention, the primary treatment phase includes days eight  
7 through twelve of the treatment cycle.

8 Alternatively, the method may comprise, either in lieu of or  
9 in addition to the mechanism of cell death via autoschizis, a  
10 mechanism of cell death via chemotherapy-induced apoptosis,  
11 however, the apoptosis cell death induced by the method of the  
12 present invention will be an immunogenic cell death, because all of  
13 the tumor cells are exposed to cellular stress prior to death.

14 At least one embodiment of the method of the present invention  
15 further comprises the administration of menadione or another  
16 pharmacological agent to potentiate the mechanism of autoschizis  
17 and/or the use of chemotherapy and/or the use of radiotherapy in  
18 combination with or as a substitute for the intravenous  
19 administration of ascorbic acid to induce the immunogenic cell  
20 death and the subsequent, immunogenic release of the plurality of  
21 TAA into the patient's system.

22 As Figure 5 further illustrates, an alternate embodiment of  
23 the method of the present invention further comprises administering  
24 the menadione to the patient during the primary treatment phase  
25 (e.g. days eight through twelve of the treatment cycle).

1 Specifically, the method of the present invention includes  
2 administering the menadione to the patient each day of the primary  
3 treatment phase at a daily dosage of approximately 250 milligrams.  
4 In one preferred embodiment, the method comprises administering the  
5 menadione to the patient intravenously, however, in at least one  
6 alternate embodiment, the menadione may be administered orally.

7 The method of the present invention further comprises  
8 providing an external vaccine to cancer patients, the external  
9 vaccine comprising a hemoderivative composition, and preferably, an  
10 autologous hemoderivative composition prepared from a blood  
11 specimen from the patient and, thus, containing at least some of  
12 the plurality of TAA released from tumor cells. In particular, the  
13 method of the present invention includes administering an external  
14 vaccine to the patient during a secondary phase of the treatment  
15 cycle. As illustrated in Figure 6, in one preferred embodiment,  
16 the external vaccine is administered to the patient on each of days  
17 fifteen, seventeen, nineteen, twenty-two, twenty-four, and twenty-  
18 six of the treatment cycle. It is well understood that numerous  
19 variations of this preferred schedule for administering the  
20 external vaccine during the secondary treatment phase are  
21 encompassed by the scope of the method of the present invention.

22 In at least one embodiment, the external vaccine is  
23 administered subcutaneously, however, it is also understood to be  
24 within the scope of the present invention to include administering  
25 the external vaccine to the patient via alternate inoculation

1 mechanisms, including, but not limited to, intradermal and  
2 intramuscular inoculations.

3 One alternate embodiment of the present invention further  
4 comprises administering cyclophosphamide to the patient each day of  
5 a second intermediate treatment phase at a daily dosage of  
6 approximately 300 milligrams per square meter of surface area of  
7 the patient's body. In at least one embodiment, the second  
8 intermediate treatment phase comprises day thirteen of the  
9 treatment cycle.

10 The present invention further comprises a method for preparing  
11 the autologous hemoderivative composition, as illustrated  
12 schematically in Figure 6, for use in eliciting an effective  
13 antitumoral immune response in a patient, such as may be utilized  
14 in the method described herein. In one preferred embodiment, the  
15 method for preparing the autologous hemoderivative composition  
16 includes extracting a blood specimen of approximately 20  
17 milliliters from a femoral artery of the patient into a first  
18 syringe pre-filled with approximately 5,000 international units  
19 (I.U.) of heparin having a concentration in a range of between  
20 approximately 250 to 300 I.U. per milliliter. The blood specimen  
21 solution is allowed to settle while maintained in vertical position  
22 at a temperature of approximately 37 degrees centigrade. After  
23 approximately one hour, an aliquot of a supernatant of white cell  
24 rich blood plasma is separated from the blood specimen solution  
25 into a second syringe containing between approximately 3 to 4 parts

1 of distilled water per part of the plasma-cell layer thereby  
2 forming a plasma-cell solution and, inducing a hypotonic cytolysis.  
3 The method of the present invention further provides that the  
4 plasma-cell solution be stored at approximately minus twenty  
5 degrees centigrade for a period of approximately 24 hours, after  
6 which, the plasma-cell solution is warmed up to approximately 37  
7 degrees centigrade in order to complete a hypotonic-hypothermic  
8 cytolysis process.

9 The resultant plasma-cell solution may be filtered through a  
10 glass wool membrane or optionally it may be centrifuged at 2000 G,  
11 in order to clear the solution and remove gross precipitates. In  
12 yet another embodiment, the resultant plasma-cell solution may be  
13 sonicated to clear the solution and remove the precipitates. The  
14 resultant plasma-cell solution is then subjected to further thermal  
15 treatment. More in particular, the method of the present invention  
16 permits utilization of any one of a plurality of thermal treatments  
17 in order to obtain different immunogens. In one preferred  
18 embodiment, the plasma-cell solution is heated to approximately 100  
19 degrees centigrade for approximately between 8 to 10 minutes,  
20 thereby forming a plasma-cell fraction. The method also provides  
21 for allowing the solution to return to room temperature,  
22 approximately 25 degrees centigrade, until temperature equilibrium  
23 is reached. Finally, the resultant plasma-cell fraction may also  
24 be either filtered through glass wool membrane, centrifuged at 2000  
25 G, or sonicated, followed by filtration through cellulose membranes



1 ranging from between approximately 0.20 to 0.45  $\mu$ m diameter.

2 While the method of the present invention for preparing an  
3 external vaccine presented above comprises one preferred  
4 embodiment, it is understood that alternative embodiments may be  
5 utilized to prepare an external vaccine comprising an autologous  
6 hemoderivative composition through modification of the methods for  
7 blood extraction, sedimentation, and/or specific temperatures and  
8 durations for thermal fractionation. In addition, it is understood  
9 that while a preferred embodiment of the present invention  
10 comprises subcutaneous inoculation of the patient with the external  
11 vaccine, inoculation via other mechanisms including, by way of  
12 example only, intradermal, intravenous, and/or intramuscular  
13 inoculation are encompassed in the method of the present invention  
14 to elicit an efficient antitumoral immune response or an  
15 antitumoral biological response targeted to tumor cells, tumor  
16 stroma patient's immune system and/or molecular mediators of the  
17 host biological response against cancer disease.

18 The present invention is also directed towards an autologous  
19 hemoderivative composition, which may be utilized as an external  
20 vaccine in the method to elicit an effective antitumoral immune  
21 response disclosed herein. In one preferred embodiment, the  
22 autologous hemoderivative composition comprises a plasma-cell  
23 solution which has been cooled to approximately minus twenty  
24 degrees centigrade for approximately 24 hours. The cooled plasma-  
25 cell solution may subsequently be heated to approximately 100

1 degrees centigrade and fractioned for between approximately 8 to 10  
2 minutes thereby forming a plasma-cell fraction, after which, the  
3 plasma-cell fraction may be filtered upon cooling, and readied to  
4 provide to the patient as an external vaccine.

5 The plasma-cell solution of the autologous hemoderivative  
6 composition may be at least partially defined by a supernatant  
7 plasma-cell layer which is separated from a blood specimen solution  
8 and a quantity of distilled water, typically, 3 to 4 parts of  
9 distilled water per part of the blood specimen solution.

10 In addition, the blood specimen solution may comprise a blood  
11 specimen extracted from a patient, preferably, from femoral artery  
12 of the patient, into a solution comprising, in at least one  
13 embodiment, approximately 5,000 international units (I.U.) of  
14 heparin at a concentration in a range of between approximately 250  
15 to 300 I.U. per milliliter.

16 Since many modifications, variations and changes in detail can  
17 be made to the described preferred embodiment of the invention, it  
18 is intended that all matters in the foregoing description and  
19 illustrated in the accompanying figures be interpreted as  
20 illustrative and not in a limiting sense. Thus, the scope of the  
21 invention should be determined by the appended claims and their  
22 legal equivalents.

23 Now that the invention has been described,  
24